THE RELATION OF NUTRIENT SALTS TO TURGOR.

EDWIN BINGHAM COPELAND.

INTRODUCTORY.

IT is now about twenty years since turgor stepped into a prominent place in vegetable physiology. At almost the same time Pfeffer[1] demonstrated the previously unsuspected heights attained by osmotic pressure, and de Vries[2] applied it to the dark problem of the dynamics of growth. The new discovery proved a scientific gold field and turgor was immediately invoked as the solvent of a variety of problems where more familiar agencies had been found inadequate. The exaggerated importance ascribed to it in growth endured almost to the present day, when the experiments of de Vries,[3] Wieler,[4] Stange,[5] Hegler,[6] Pfeffer,[7] Schwendener and Krabbe,[8] Kolkwitz,[9] and Copeland[10] have shown that it cannot supply the energy necessary for growth (Pfeffer), that growth can occur without turgor stretching (Pfeffer, Kolkwitz), and that abnormally slow growth is more

[1] Osmotische Untersuchungen. 1877.

[2] Untersuchungen über die mechanischen Ursachen der Zellstreckung. Leipzig. 1877.

[3] Eine Methode zur Analyse der Turgorkraft. Jahrb f. wiss. Botanik **14**: 427. See also page 561.

[4] Berichte d. deutschen bot. Gesell. **5**: 375. 1887.

[5] Beziehungen zwischen Substratconcentration, Turgor, und Wachsthum bei einigen phanerogamen Pflanzen. Bot. Zeit. **50**: 253. 1892.

[6] Über den Einfluss des mechanischen Zugs auf das Wachsthum der Pflanze. Beiträge zur Biologie d. Pflanze **6**: 382. 1893.

[7] Druck- u. Arbeitsleistung durch wachsende Pflanzen. Leipzig. 1893.

[8] Über die Beziehungen zwischen dem Maass der Turgordehnung und der Geschwindigkeit der Längenzunahme wachsender Organe. Jahrb. für wiss. Botanik **25**: 323.

[9] Untersuchungen über Plasmolyse, Elasticität, Dehnung, und Wachsthum, an lebendem Markgewebe. Fünfstück's Beiträge. 1895.

[10] Über den Einfluss von Licht und Temperatur auf den Turgor. Halle a. S. 1896.

likely to increase, and abnormally rapid growth to decrease the turgor, than to be caused by variation in the latter.

In Stange's work, the substratum was merely a physical agent of varying concentration. But few salts were used, and no essential difference was observed in their action. Beyond a suggestion by Benecke[11], that the turgor may be caused by the presence of sodium as well as by that of potassium, and that the continual acquisition of the former probably serves such "secondary" functions, the relation between the chemical nature of the substratum and the turgor has never been considered. To assist in clearing this untrodden field, my investigation was undertaken.

It was hoped in beginning this study that a considerable number of chemical elements would be made to show some direct influence on turgor. But as the tables well show, this hope was not realized. With the exception of potassium, and in one case, perhaps of NO_3, the removal of any food constituent did not tend to depress the turgor, or if it did, it stopped the growth so quickly and effectively that the turgor rose. Failure to get evidence on the question in hand is then added support for the thesis already referred to,[12] that growth regulates turgor more decidedly than turgor growth.

Methods.—All the plants used as subjects of experiment were grown in water culture, in glass jars, protected from the light by heavy drying paper. The covers of the jars were wood, bored nearly through with a large auger, the rest of the way with a small one, furnishing an excellent support for the seeds. The jars for most of the experiments were of a little less than three liters capacity, larger ones of eight liters being sometimes used. During the time that the experiments lasted,

[11] Ein Beitrag zur mineralischen Nahrung der Pflanzen. Berichte d. deutschen bot. Gesell. 1894. Generalversammlung, p. 114. "Es ist absolut nicht einzusehen, warum nicht in solchen Leistungen, die weniger eng an vitale Functionen gekettet, mehr als formale Bedingungen des Lebens aufzufassen sind—nahe liegt es hier, z. B. an osmotische Leistungen zu denken—z. B. Natriummolekel für das Kalium einspringen könnten.

[12] Copeland, loc. cit.

the larger size seemed unnecessary, and was much less convenient. The seeds were germinated in clean sawdust, and when the radicles were a few centimeters in length, average specimens were selected from a large number, placed in the culture jars, and plugged fast with cotton. When the duration of the experiment demanded it, the solutions were renewed, but in many cases it sufficed to keep the jars full by occasional addition of distilled water.

The first solutions were prepared according to de Vries' isotonic coefficients [13]: afterward they were made according to molecular equivalents. The latter is the more scientific plan, but does not differ enough to affect the results. All salts used were of absolute purity, and the utmost care was taken that the concentration was exactly that stated: for instance, in making up $MgSO_4$ solution all dehydrated crystals were removed individually before the salt was weighed; and such compounds as $Ca(NO_3)_2$, which cannot well be weighed dry, were made in solution synthetically by the writer. Clarke's atomic weight determinations were used. Making up the solutions by exact molecular equivalents secured as nearly as was possible their osmotic equality. For the individual salts, in solution alone, this might have been accomplished more accurately by taking into account their varying degrees of dissociation. But as the opportunity for unavoidable and unseen breaking up and reformation of molecules in such dilute solutions is limited only by the variety of salts dissolved, there is no way of determining the salts whose dissociation should be reckoned with. At any rate, in solutions so dilute as those used dissociation of all nutrient salts is very complete, and in practice may be regarded as quite so.

The turgor was determined by the usual method, *i. e.*, by immersing sections in a sequence of solutions of known strength of KNO_3. In the experience of the writer, no other solvent has proved itself so available for this purpose as saltpeter. It is easily and accurately prepared, keeps well, diffuses quickly

[13] Op. cit.

through the plant, and is harmless for any reasonable duration of experiment. It has, however, one theoretical disadvantage which has hitherto been overlooked, namely that, like any other crystalline salt, it dissociates in solution, and to an increasing extent with progressive dilution. Any given solution is then a little more than half as strong osmotically as one with twice the weight of dissolved saltpeter.[14] This objection might be overcome by making solutions according to the osmotic instead of per cent. strength, but since this source of error is less than that arising from the inaccuracy of the tests and the individual variations of the plants and the cells, and the correction at best would only be relative, it seemed best to conform to the established method. In the reports of the experiments the expression "turgor = 2.5 per cent." means that 2.5 per cent. KNO_3 is just sufficient to begin plasmolysis.

THE EXPERIMENTS.

Phaseolus multiflorus. Two series of cultures were carried through. The complete or standard "normal" solution was made by mixing, and diluting to one-twentieth, equal parts of the following solutions, prepared according to de Vries' isotonic coefficients:

$Ca(NO_3)_2$	9.876 %	isotonic to	0.8 æq. KNO_3
KNO_3	2.525 %	or	0.25 æq.
NaCl	1.462 %	isotonic to	0.25 æq. KNO_3
K_2HPO_4	2.597 %	" "	0.25 æq. KNO_3
$MgSO_4$	4.464 %	" "	0.25 æq. KNO_3

A. Put in cultures November 23. Radicles 3–5^{cm} long.

1. In normal solution.

		Nov. 28	Jan. 5	Feb. 13
Stem	length	11^{cm}	50^{cm}	50^{cm}
	turgor	2.5 %	2.5–3 %	2.5–3 %
Root	length	10^{cm}	——	55^{cm}
	turgor	2–2.5 %	2 %	2 %

[14] A 5 per cent. solution is only 4.67 times as strong osmotically as a 1 per cent. solution. Kohlrausch, Wied. Ann. **26**: 195.

2. In distilled water.

Stem	length	5^{cm}	33^{cm}	mostly dead.
	turgor	2–2.5 %	3 %	3 %
Root	length	6.5^{cm}	——	dead.
	turgor	2 %	2 %	

3. With $NaNO_3$ and Na_2HPO_4 instead of KNO_3 and K_3HPO_4, in equivalent quantities.

Stem	length	9^{cm}	50^{cm}	44^{cm}
	turgor	2.5 %	2 %	2 %
Root	length	9.5^{cm}	——	20^{cm}
	turgor	2.5 %	1.5–2 %	1.5 %

The solutions were renewed once during the experiment, and at its close the plants in complete solution were growing nicely. The plants tested January 5 were the largest in all the jars.

B. Put in water cultures November 30.

1. Normal.

		Dec. 17	Jan. 12	Jan. 25 (Temp. down to 10°C.
Stem	length	26^{cm}	42^{cm}	45^{cm}
	turgor	2.5 %	2.5–3 %	3–3.5 %
Root	length	19^{cm}	——	——
	turgor	2–2.5 %	2 %	2.5 %

2. Distilled H_2O.

Stem	length	7^{cm}	19^{cm}	——
	turgor	2–2.5 %	2.5–3 %	2.5 %
Root	length	6^{cm}	8^{cm}	——
	turgor	2 %	1.5 %	2 %

3. Na instead of K.

Stem	length	30^{cm}	52^{cm}	65^{cm}
	turgor	2 %	2 %	2.5 %?
Root	length	19^{cm}	——	nearly dead.
	turgor	2 %	1.5 %	2 %

4. With $CaCl_2$ and KCl instead of $Ca(NO_3)_2$ and KNO_3.

Stem	length	20^{cm}	dead.
	turgor	2.5 %	
Root	length	14^{cm}	——
	turgor	2 %	2 %

5. With $NaNO_3$ instead of $Ca(NO_3)_2$ ("isotonic" quantities).

Stem	length	25cm	26cm	29cm
	turgor	2.5 %	3 %	3 %
Root	length	18cm	——	22cm
	turgor	2 %	2 %	2–2.5 %

The beans without K flourished as well as those with it until almost the end of the experiment. As the plants become older and irregularly branched, and in many cases the main root dies and is replaced by branches, the length ceases to be a measure of the growth. But wherever it was any fair test it is included in the tables, so that the unquestionable influence of the growth on the turgor may be apparent. The cotyledons of seedlings in the last two cultures (4 and 5) were emptied more slowly than those of the others, which would tend to keep down the turgor of the growing parts. Occasional tests of the leaves of 1 and 3 showed their turgor to agree at about 3.5 per cent.

Phaseolus vulgaris. Seedlings put in the solutions December 9.

1. Normal.

		Jan. 9	Jan. 18	Jan. 27	Feb. 27
Stem	length	19cm	23cm		
	turgor	2.5 %	2–2.5 %	3 %	3 %
Root	length	9cm	10cm		
	turgor	2 %	1.5–2 %	2 %	2 %

2. Distilled H_2O.

Stem	length	10cm	(tip dead)	dead.
	turgor	2.5–3 %	2.5–3 %	
Root	length	8cm	8cm	dead.
	turgor	2 %	2 %	

3. Na instead of K.

Stem	length	19cm	25cm	27cm	
	turgor	2 %	2–2.5 %	2.5 %	2.5–3 %
Root	length	19cm	22cm		
	turgor	1.5 %	1.5–2 %	1.5 %	2 %

4. Cl instead of NO_3.

Stem	length	16cm	17cm	
	turgor	2–2.5 %	2.5 %	2.5–3 %
Root	length	10cm	12cm	dead.
	turgor	2 %	2 %	

5. Na instead of Ca.

Stem	length	12cm	14cm	16cm	dead.
	turgor	2.5%	2.5%	2.5–3%	
Root	length	5cm	7cm	10cm	dead.
	turgor	2%	2%	2%	

The leaves of 1, 3, and 5 were tested January 18, and all plasmolyzed in 3.5 per cent. The plants in the complete culture were of unhealthy appearance January 27, the leaves being spotted with brown dry flecks, and the roots short and bushy. Culture 3 showed the same symptoms in milder form. The solutions were poured off and replaced with others prepared from fresh salts (see below under Pisum for composition), and a better condition ensued. The seedlings in NO_3-free solution bore very empty and colorless roots, which soon rotted; while those deprived of Ca, though dwarfed, were rugged and hardy in appearance. The power of the Leguminosæ to endure want of K better than that of Ca[15] was well illustrated by both species of Phaseolus.

Pisum sativum. Placed in solution March 13. The normal solution used in this and most of the following experiments was:

$Ca(NO_3)_2$,	0.006 æq.
KCl,	0.0025 æq.
K_2HPO_4,	0.0015 æq.
$MgSO_4$,	0.0025 æq.

With a trace of ferric chloride in the jars, as needed.

The course of turgor and growth was:

1. Normal.

		March 27	April 13	May 4
Stem	length	34cm	37cm	
	turgor	2.5%	3%	2.5%
Root	length	25cm	26cm	
	turgor	2%	1.5–2%	2%

2. Distilled water.

Stem	length	22cm	30cm	
	turgor	2%	2%	2%
Root	length	12cm	25cm	
	turgor	2%	1–1.5%	1.5%

[15] Cf. Koenig u. E. Haselhoff: Die Aufnahme der Nährstoffe aus dem Boden durch die Pflanzen. Landw. Jahrb. 23: 1009.

3. KCl and K_2HPO_4 replaced by equivalent quantities of NaCl and Na_2HPO_4.

Stem	length	27cm	34cm	
	turgor	2–2.5%	2.5%	2.5%
Root	length	23cm	29cm	
	turgor	1.5–2%	1.5%	1.5%

4. $CaSO_4$ instead of $Ca(NO_3)_2$.

Stem	length	26cm	35cm	
	turgor	2.5%	2.5–3%	2.5–3%
Root	length	26cm	26cm	
	turgor	2%	2%	2%

The substitution of $CaSO_4$ for its molecular equivalent $Ca(NO_3)_2$ involves a slight reduction of the osmotic power of the solution. This, taken alone, would tend to lower the turgor (Stange), but it was unable to make itself felt in the result, for the turgor of these stems (4) was the highest in any solution at the close of the experiment.

Sinapis alba. Seedlings were placed in solution, such as were used for Phaseolus, December 9. This plant is not adapted to water culture, and the specimems were never vigorous, though none of them in 1 or 3 died during the experiment.

1. Normal.

		Dec. 21	Jan. 23	Feb. 8
Stem	length	5cm	5.5cm	7cm
	turgor	3%	3%	4%

2. Distilled H_2O.

Stem	length	4cm	dead
	turgor	2%	

3. Na instead of K.

Stem	length	4.5cm	5cm	5.5cm
	turgor	2%	2.5%	3%

The roots were stunted from the start, and the unusual height of the turgor of the stems may have been due to the accumulation of food, at least in part. But starch was present in 3, as well as in 1, so that their difference in turgor (1 per cent. at the close of the experiment) must be referred to the effect of the presence or absence of K, for a reasonably definite

and not too unequal quantity of sugar must have preceded the formation of starch in both cases.

Fagopyrum, the Japanese buckwheat. The normal culture was like that used for Pisum. In the table, which in this case is a compilation, the length of the parts is omitted. In relative thriftiness in the different solutions the plants agreed quite well with those grown by Nobbe[16] in his classic work on the plant's need of potassium. The time is measured from the dates when the seeds were put in water culture.

	After 9 days	After 19 days	After 33 days	After 42 days	After 57 days
1. Normal.					
Stem	2–2.5%	2–2.5%	2.5%	2.5%	2.5–3%
Root	2%	2%	2%	2%	
2. Distilled H_2O.					
Stem	1.5%	2%	2%		
Root	1.5%	1.5%	1–1.5%		
3. Na instead of K.					
Stem	2%	2%	2%	2%	2–2.5%
Root	1.5%	1.5–2%	1.5%	1.5%	
4. $Ca(NO_3)_2$ replaced by $CaSO_4$.					
Stem		1.5–2%	2%	2%	
Root		1.5%	1.5–2%		
5. $Ca(NO_3)_2$ replaced by $NaNO_3$.					
Stem	2–2.5%	2.5%	2.5–3%	3%	3%
Root	2%	2%	2%		
6. $MgSO_4$ replaced by $MgCl_2$					
Stem	2.5%	2.5%	3%	3.5%	
Root	2%	2–2.5%	2–2.5%		
7. $MgSO_4$ replaced by $CaSO_4$					
Stem		2.5%	3%		
Root		2–2.5%	2–2.5%?		
8. K_2HPO_4 replaced by K_2SO_4.					
Stem		2.5%	3–3.5%	3.5%	
Root		2%	2–2.5%		

With the substitution of $MgCl_2$ for $MgSO_4$ occurred a small increase in the osmotic power of the solution, which, however,

[16] F. Nobbe, Landw. Versuchsst. 13 : 369.

cannot suffice to explain the very high turgor of the plants grown in it (6). When $Ca(NO_3)_2$ was replaced by $CaSO_4$ the osmotic power was reduced, but here, too, the turgor surpassed the normal.

The results given in this table tally very well with those obtained on Phaseolus, Pisum, and Sinapis, as far as the latter were carried out, the only difference being in the low turgor of buckwheat grown in cultures free of nitrates. In another series the turgor of the stems grown in nitrate-free cultures rose to 2–2.5 per cent., while in normal stems it was 2.5 per cent., both tested before there was any marked difference in growth. It certainly looks as though the turgor fell because the nitrates were absent, but no such tendency could be detected in any plant tested except the buckwheat. The turgor of 5 was distinctly higher than that of 1 before any difference was noticeable in their growth. The last three cultures (6, 7, and 8) were stunted in growth by the end of the second week, which explains their extreme turgor.

Zea Mays. Two series of cultures were carried through. For the first, dating from January 16, the cultures were made as described under Phaseolus.

1. Normal.

		Feb. 6	Feb. 18	March 6	March 13	April 13
Stem	turgor	2.5%	2.5%	3%	3–3.5%	
Root	length	36cm	48cm	60cm	60cm	
	turgor	2%	2%	2%	2–2.5%	

2. Distilled H_2O.

Stem	turgor	1.5–2%	2–2.5%	1.5–2%	1.5%	1.5–2%
Root	length	29cm	34cm	47cm		50cm
	turgor	1.5%	1.5%	1–1.5%	1.5%	1–1.5%

3. K replaced by Na.

Stem	turgor	2%	2–2.5%	2.5%	2.5%
Root	length	32cm	38cm	41cm	43cm
	turgor	1.5–2%	2%	2%	1.5–2%

4. Ca replaced by Na.

Stem	turgor	3%	3%	3.5%	dead
Root	length	34cm	36cm	54cm	dead
	turgor	2%	2%	2–2.5%	

The normal solution for the second series, beginning March 11, was that given under *Pisum sativum*.

1. Normal.

		Mar. 22	Apr. 2	Apr. 21	May 5	June 11
Stem	length	22cm	35cm	52cm	75cm	85cm
	turgor	2%	2%	2%	2.5%	3%
Root	length	36cm	41cm	66cm		76cm
	turgor	2%	1.5–2%	2%	1.5–2%	2%

1a. Containing 0.01 æq. instead of 0.0025 æq. $MgSO_4$.

Stem	length	21cm	34cm	50cm	61cm	dead.
	turgor	2%	2.5%	2.5%	2.5–3%	
Root	length	31cm	34cm	41cm	47cm	dead.
	turgor	2%	2%	2%	2%	

2. Distilled H_2O.

Stem	length	17cm	26cm	32cm	34cm	35cm
	turgor	1.5–2%	1.5%	1.5%	1.5%	2.5%?
Root	length	46cm	75cm	80cm	85cm	unsound
	turgor	1.5%	1–1.5%	1–1.5%	1.5%	1.5–2%

3. K replaced by Na.

Stem	length	20cm	26cm	32cm	32cm	dead?
	turgor	1.5–2%	1.5%	1.5–2%	1.5%	
Root	length	34cm	45cm	54cm	56cm	dead.
	turgor	1.5%	1.5%	1.5%	1.5%	

3a. KCl replaced by NaCl.

Stem	length	19cm	28cm	37cm		
	turgor	1.5–2%	2%	2%	2%	2.5–3%
Root	length	26cm	35cm	43cm		
	turgor	1.5–2%	1.5–2%	1.5–2%	1.5%	2%

3b. K_2HPO_4 replaced by Na_2HPO_4.

Stem	length	21cm	31cm	44cm	50cm	
	turgor	1.5–2%	2%	2–2.5%	2–2.5%	2.5–3%
Root	length	30cm	39cm	50cm		
	turgor	1.5–2%	2%	2%	2%	2%

3c. 0.005 æq. instead of 0.0025 æq. KCl: 0.003 æq. instead of 0.0015 æq K_2HPO_4

Stem	length	20cm	28cm	41cm	51cm	
	turgor	1.5–2%	2.5%	2.5–3%	2.5%	3%
Root	length	36cm	40cm	64cm		
	turgor	1.5–2%	1.5–2%	2%	1.5–2%	2%

4. $CaSO_4$ instead of $Ca(No_3)_2$.

Stem	length	21cm	30cm	34cm	40cm
	turgor	2%	2–2.5%	2.5%	3–3.5%
Root	length	40cm	52cm	103cm	110cm
	turgor	1.5–2%	2%	1.5–2%	2%

5. K_2SO_4 instead of K_2HPO_4.

Stem	length	24cm	34cm	41cm	47cm	
	turgor	2%	2%	2.5%	3%	3.5%
Root	length	31cm	38cm	67cm	85cm	
	turgor	2%	2%	2%	2%	2–2.5%

In measuring the "stem" (Spross) the average height of the longest leaves of all the plants in the culture was taken, hence the growth appears to have been more uniform than in any of the preceding tables, in which the length is computed only from the plants used at each time for the tests. The turgor of the "stem" (Stengel) was determined in the upper part of one of the older internodes, at a point where growth was assumed to have ceased.

The difference in turgor between 1 and 1a would probably have been produced by the addition of the same quantity (0.0075 æq.) of any harmless salt, the action being probably purely physical. The cultures 3, 3a, 3b, and 1 form a series in which the K gradually increases without any change in the osmotic strength. The result shows a strikingly uniform gradation in both growth and turgor. Up to about the point of the normal solution an increase in the relative amount of K present was certainly beneficial to the plants, but doubling the K then present (3c) may not have had any more effect on the growth, or the turgor, than the addition of the same quantity of another salt (compare 1a). At the close of the experiment the only thrifty plants were in cultures 1 and 3c. All the plants in 1a, 3, and 4 were dead, and those in 3a and 3b had only the younger leaves and roots still making a feeble show of life.

CONCLUSIONS.

The testimony of the different experiments is so uniform, and the conclusions to be drawn are so simple and manifest,

that they have been reserved for discussion until after all of the tables have been presented.

1. Potassium presented in solution to the roots of plants causes the cells of both root and stem to exhibit a higher turgor than they can do when it is replaced by sodium. There is no conceivable reason for suspecting the Na of depressing the turgor; in fact the "normal" solution in some experiments contained Na, and in others did not, without any difference appearing in the results. So we may state it, that *potassium is a factor*, direct or indirect, *in the turgor of the plant.*

2. *There is no experimental ground for attaching this significance to any other constituent of the mineral food.*[17]

It need hardly be stated here that, in common with any molecule of any kind dissolved in the cell sap, the elements if present in solution must contribute to the turgor. Nor is it impossible that they are sometimes important factors in it; but if they are, our methods cannot demonstrate it. In some instances, as when plants in an incomplete solution are able for a time to grow normally and to maintain at least a normal degree of turgor, we can say decidedly that their presence does not contribute to the turgor. Fagopyrum and Zea grown without Ca, and Zea without HPO_4 illustrate the case against these two food elements. Again, when in the absence of some food constituent the depressed growth was able to produce a very high turgor, it is improbable that the substance in question is ever a considerable part of the plant's osmotically active matter. HPO_4, Mg, and SO_4 are such substances, whose absence was accompanied by low growth and high turgor. When the absence of K, on the other hand, stunted the growth (compare 1 and 3 of Zea) the turgor still remained low.

The behavior of the various plants grown in distilled water is worthy of more than the passing notice it can here receive. Many plants will grow very rapidly in pure water until their stored-up food is exhausted. In consequence at once of the rapid growth and of the want of nourishment they have a low tur-

[17] The food product of assimilation might usually be added to this list.

gor which must remain low even when their growth stops before death. But in other cases they fall behind their better fed rivals while their reserve food is still far from used up, as though the distilled water were in itself an active agent in restricting growth, and the turgor of these plants not uncommonly becomes as high as that of those normally grown (*Phaseolus vulgaris*). Such seedlings will sometimes become accustomed to the water, and after once ceasing to elongate will put forth a luxuriant new growth. As a sample of a number of similar cases a seedling of Zea may serve, whose roots were 13^{cm} long February 10; February 19 they were still 13^{cm}; but a week later they began to grow, and by March 10 measured 30^{cm}. The roots of the first growth were remarkably thick and coarse, and but little branched; the later ones almost capillary and mesh-forming.

Except for a few plants, and a very few salts, the growth of plants in water cultures has never been studied, and will well repay a comprehensive, painstaking, comparative investigation.

Is the influence of K upon the turgor a direct one? That is, does the K itself enter into the cell sap, in whatever combination, in such quantity as to be a considerable part of the matter in solution there, and is Na unable to replace the K in this physical function? Or is it by its activity in the protoplasm that K regulates the accumulation of other substances in the sap, in which respect Na, relatively inert and unessential to the plant, could not be expected to be equally effective? At first sight, especially as K is held to play a prominent part in both the production and the translocation of the carbohydrates, one is inclined to regard the indirect course as the true one. To settle the question, seedlings of *Phaseolus multiflorus* were put into water cultures in two ten-liter jars, one containing the normal mixture of $Ca(NO_3)_2$, KCl, K_2HPO_4, and $MgSO_4$, already given under Pisum, the other different only in the substitution of Na for K. The jars were kept nearly full by addition of distilled water until June 3, during which interval the K fed plants transpired about three liters more water than did the others. The "crop" was then harvested, the roots quickly dried with filter paper,

the leaves and younger growing stems removed, and the stems and roots separated and bottled. The average turgor at the time was :

In K solution : roots, 1.9% ; stems, 3.6%.
In Na solution : roots, 1.6% ; stems, 2.9%.

The excess of turgor in the K plants was then about 0.3% in the roots, and 0.7% in the stems. Abundant starch was present in all the stems of both cultures. By throwing away the leaves and youngest internodes, and separating the stems and roots, material was obtained for each analysis whose turgor was known and uniform. Seedlings of *Zea Mays* were grown in the same way from April 10 until June 12. Two of the K and several of the Na plants were then dead. The roots, especially of the latter, were in bad condition, while those grown with K were very thrifty. All dead parts and the blades of the leaves were removed, and the stems and roots separated and bottled. The average turgor was :

In K solution : roots, 2.2% ; stems, 3.6%.
In Na solution : roots, 1.8% ; stems, 2.0%.
Excess of K over that in Na solution : roots, 0.4% ; stems, 1.6%.

As soon as the material from each culture had been gathered, it was corked tightly in a bottle to prevent egress or ingress of watery vapor, and heated to 120°C. in the autoclave.[18] It was then cooled and all the sap immediately pressed out, filtered, and measured. When the sap did not exceed 20^{cc}, it was all used for analysis. The analysis consisted in oxidizing and removing all organic matter by repeated boiling to dryness with HNO_3 and $HNO_3 + HCl$; filtering and washing residue until no more Cl is dissolved ; addition of NH_4OH, which caused no cloudiness ; removal of Mg and any other heavy metals by excess of BaOH ; removal of Ba and Ca by precipitation with $(NH_4)_2CO_3$; evaporation to dryness and heating to a white heat ; and cooling and weighing. The residue should be entirely soluble KCl and NaCl. For the sake of certainty it

[18] For justification of killing the plants, see deVries, loc. cit.

was refiltered and again dried and weighed. The weight of KCl + NaCl being known, the KCl was removed with $PtCl_4$, the K determined, and the amount of Na found by subtraction.

The statistical results are embodied in the accompanying table, compiled according to Meyer and Seubert's atomic weights.

	Vol. of sap analyzed	KCl + NaCl		KCl		NaCl		K + Na
		Weight	%	%	Equivalence	%	Equivalence	Equivalence
PHASEOLUS:								
K Stem	20cc	0.187 g	0.935	0.7078	0.0931	0.2277	0.0391	0.1342
K Root	16	0.0777	0.4856	0.3731	0.0504	0.1125	0.0193	0.0697
Na Stem	12.5	0.0421	0.3368	0.2184	0.0294	0.1184	0.0203	0.0497
Na Root	16	0.072	0.450	0.1038	0.0138	0.3462	0.0593	0.0731
ZEA:								
K Stem	20cc	0.159 g	0.795	0.6064	0.0815	0.1785	0.0306	0.1121
K Root	20	0.109	0.545	0.4005	0.0538	0.1445	0.0248	0.0786
Na Stem	10	0.0206	0.206	0.0828	0.0111	0.1232	0.0211	0.0322
Na Root	3.8	0.010	0.263	0.1187	0.0160	0.1445	0.0248	0.0840

The weight per cent. of K and Na was calculated, but is omitted from the table. Comparison with v.Wolff's analyses [19] makes it appear probable that both elements are more abundant in the cell-sap than in the plant as a whole. Still this appearance may be due entirely to different conditions of growth. In the table, K and Na are represented in the form of chlorides merely for the sake of convenience, since analysis yielded them in that form. As both K and Na were present to some extent in all the sap analyzed, the sum of the two is the most suitable character by which to compare the cultures. This sum, of course, should be measured, not by the percentage weight, but by the gram-molecule (the equivalence). Multiplying the figures in the last vertical column by ten will give very nearly the osmotic power of the several mixtures of K and Na salts, measured in per cent. of KNO_3.

The difference in the aggregate of salts of K and Na between the stems of Phaseolus grown in the K- and those in the Na-

[19] Condensed in Ad. Mayer: Ernährung der grünen Gewächse, 307. Heidelberg, 1895.

bearing solutions is sufficient to create a difference in the turgor of 0.85 per cent., while the difference actually observed was only 0.7 per cent. This was exceptional, but in general the variation in the sum of the alkali metals followed that in the turgor quite satisfactorily. Only in the roots of Phaseolus did this fail. In these the per cent. of KCl+NaCl was greater in the K cultures, but the greater proportion of the lighter salt makes the mixture really more concentrated in the Na plants. The excessive turgor of the stems of Zea in the K solution (4%) was too much to explain by the accumulation of potassium. Still their sap contained 3.5 times as much of KCl+NaCl as did that of the Na plants. The stems of both Phaseolus and Zea grown in Na culture were strikingly weak in K+Na, much more effected in this respect than were the roots by the supplanting of the potassium, the plant appearing less able to make its preference felt in the organs in immediate contact with the substratum.

To make this table perfect to the chemist's eye a correction would have to be introduced for the degree of dissociation. It is said that the most completely dissociated of all salts are those of sodium. If this be true, the combined osmotic power varies a very little more widely than does the combined equivalence from the sum of the per cents. of Na and K present in any mixture. Assuming still, however, that K and Na are present as chlorides, the available data point in the opposite direction, for Kohlrausch's[20] original determinations of electrical conductivity and Landolt and Börnstein's compilation, based partly on newer figures from Kohlrausch, both represent the potassium chloride as the more highly dissociated.

From the analysis of the sap we must conclude then that *the influence of potassium on the turgor of the plant is direct.* When offered to the roots it is taken up and stored in the cell-sap, where it becomes an important part of the osmotically active material which keeps the cell and plant turgid. This function it does not share with sodium. Well known as is the useless-

[20] Ueber das Leitungsvermögen einiger electrolyte in aensserst verdünnter wässriger Lösung. Wied. Ann. **26**: 161 (p. 195).

ness to the plant of sodium as a chemical, this conclusion is still surprising. The phenomenon of turgescence is explicable by purely physical laws, and is dependent on the vitality of the plant only for the semi-permeable membrane, the living protoplasm differentiated as "Hautschicht." And yet, here where the physical properties of sodium would seem to give it a distinct advantage over the heavier potassium, leaving out of consideration the relative scarcity of the latter, we find the plant's insistence on potassium as decided as it is for the vital processes of growth and photosyntax. Indeed, in many instances (Phaseolus, Pisum) the effect of want of potassium appears in the turgor, while growth still continues normal. The plant can exhaust to the last trace the potassium of its substratum, yet it was found in appreciable quantity in the cell-sap of plants whose protoplasm was dying for want of it; as Zea (3a and 3b) after a large part of the plant was brown and dry still showed its highest turgor in the parts still living. Though the living plasma may bound itself toward the cell sap with the same "Plasmahaut"[21] which it opposes on the other side to its environment, yet the vacuole is physiologically as truly a vital part of the cell as is the alimentary canal an essential part of the human being.

The laboratory work whose results are embodied in this paper was performed at the University of Wisconsin in the laboratories of Professor Barnes and Dr. Kahlenberg, to whom I wish to express my sense of obligation for the admirable equipment placed at my disposal, and for their kindly assistance at all times.

UNIVERSITY OF INDIANA, BLOOMINGTON.

[21] W. Pfeffer: Zur Kenntniss der Plasmahaut und der Vacuolen, etc. Leipzig, 1890.